

FIG.1

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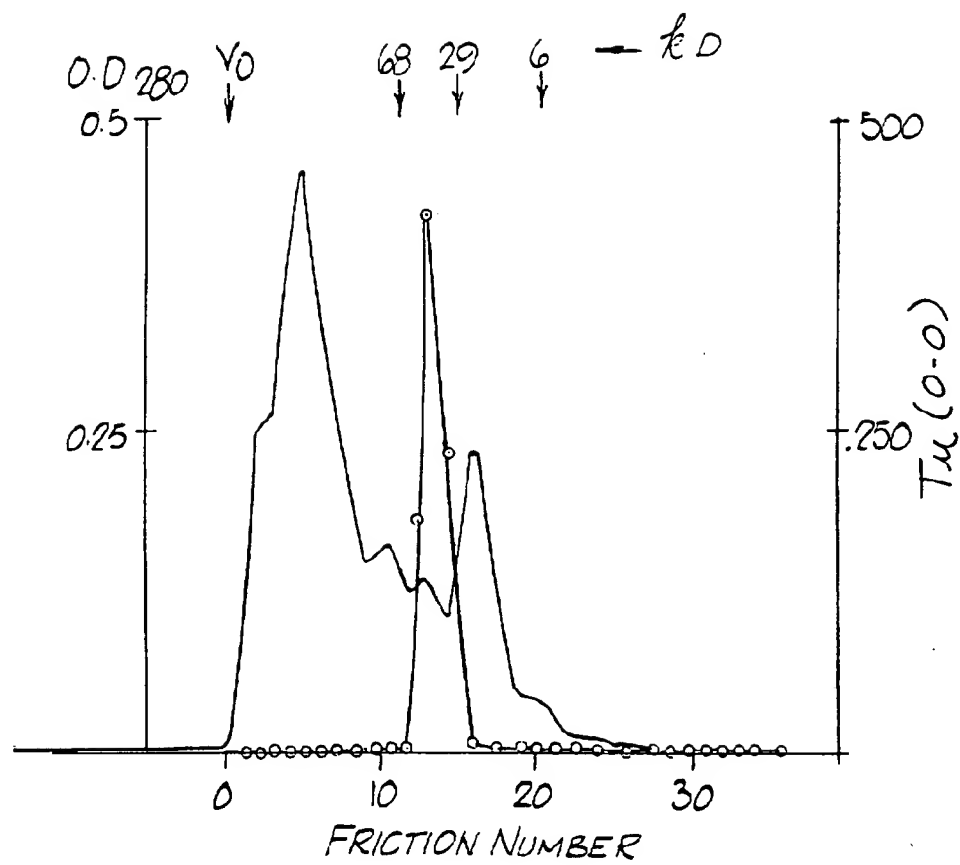


FIG. 2

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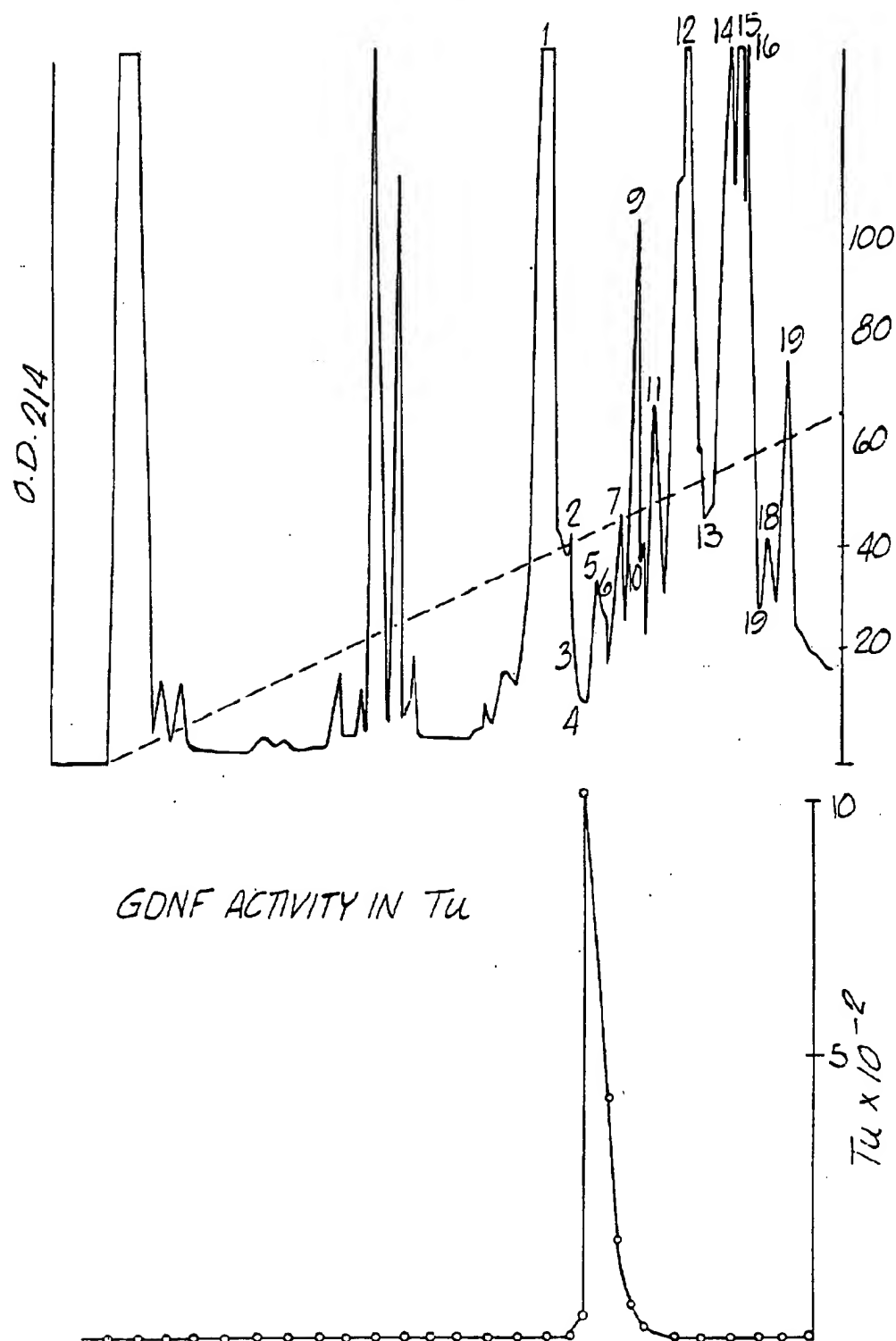


FIG. 3  
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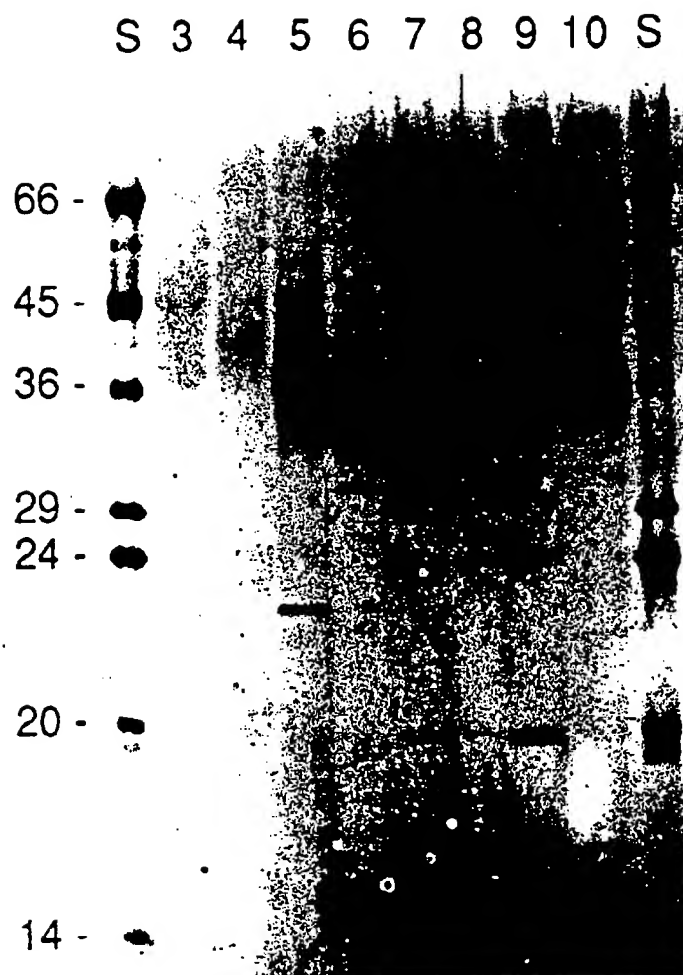


FIG. 4

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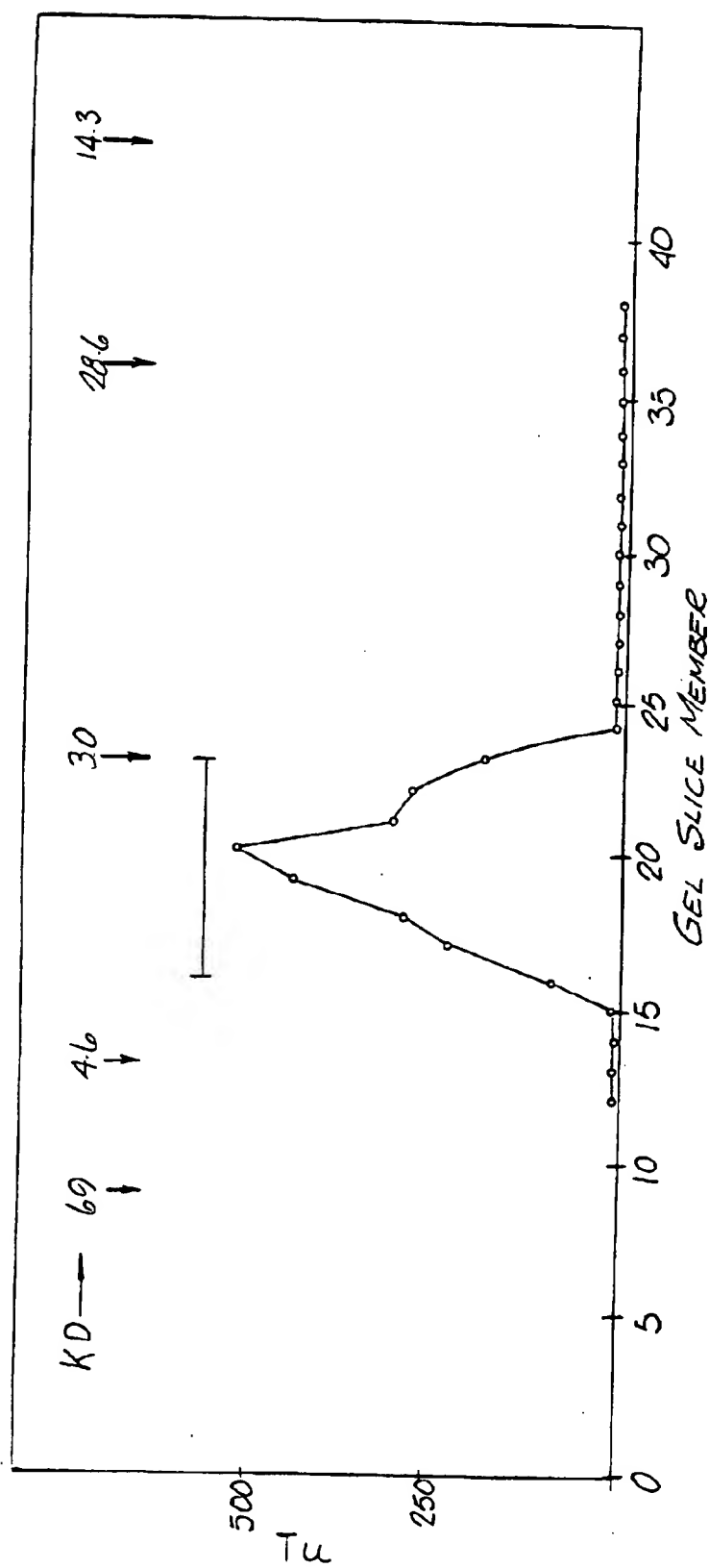


FIG.5

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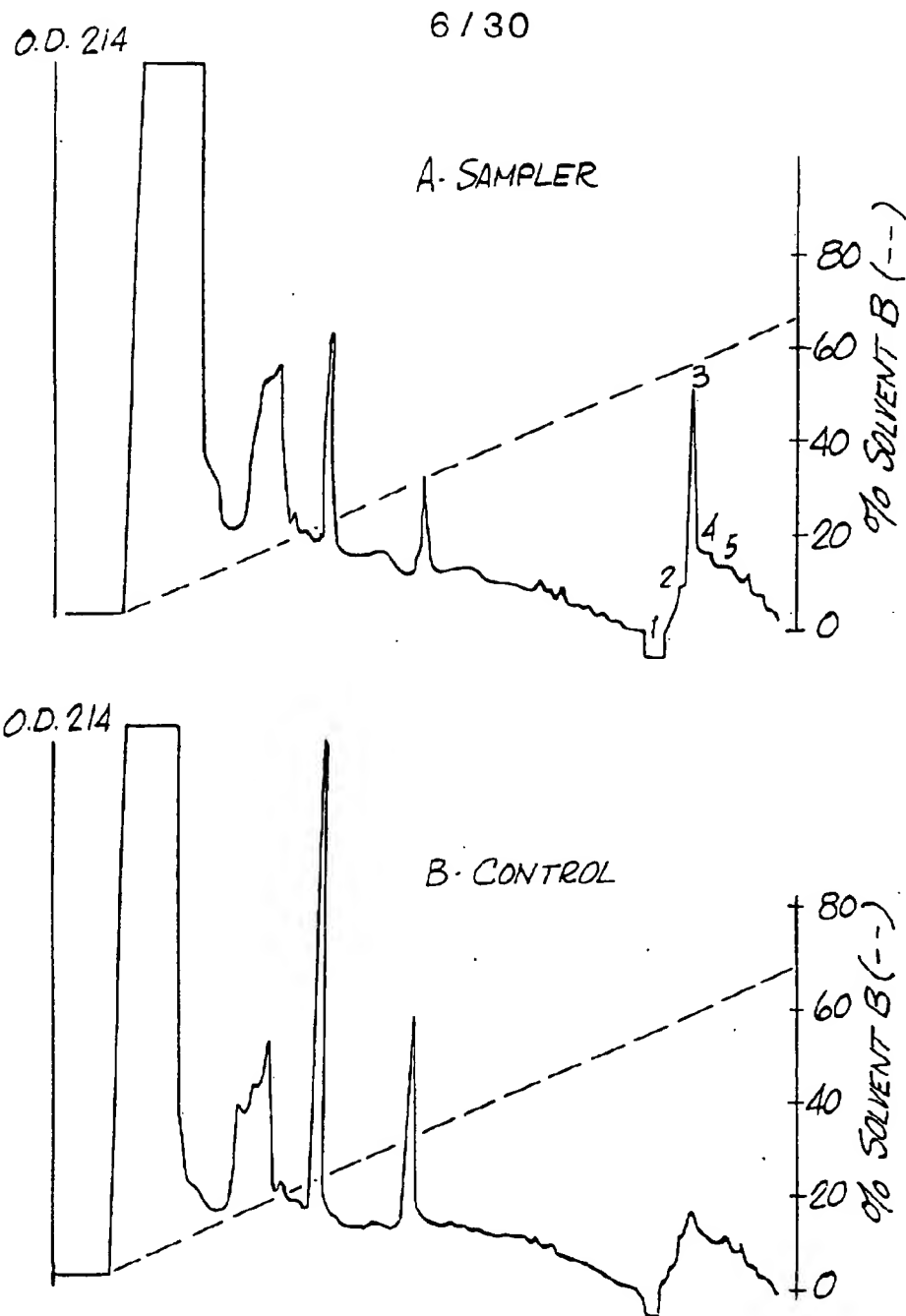


FIG. 6

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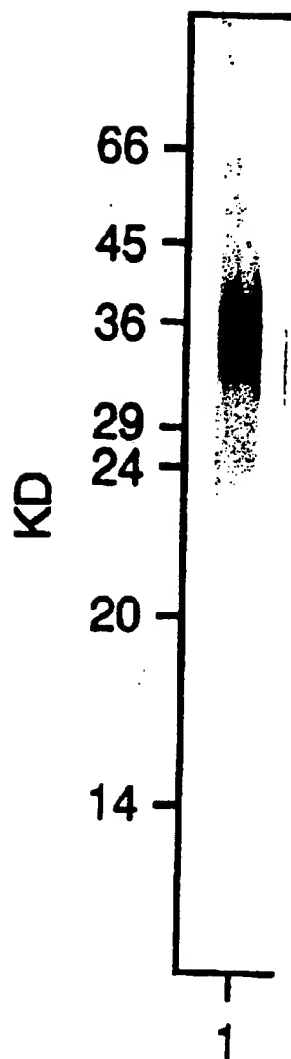


FIG. 7

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## FIGURE 8

Amino-terminal sequence of GDNF

(Ser)-Pro-Asp-Lys-Gln-Ala-Ala-Ala-Leu-Pro-Arg-Arg-Glu-  
(Arg)-Asn-( )-Gln-Ala-Ala-Ala-(Ser)-Pro-(Asp)-(Asn)

no residue could be unequivocally identified in this position

amino acid residues in parenthesis are those identified with less certainty

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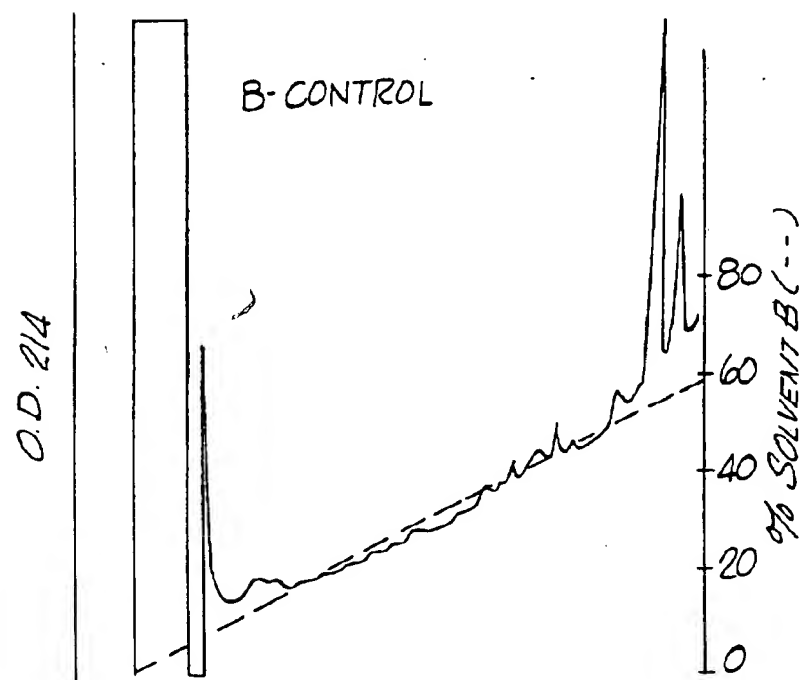
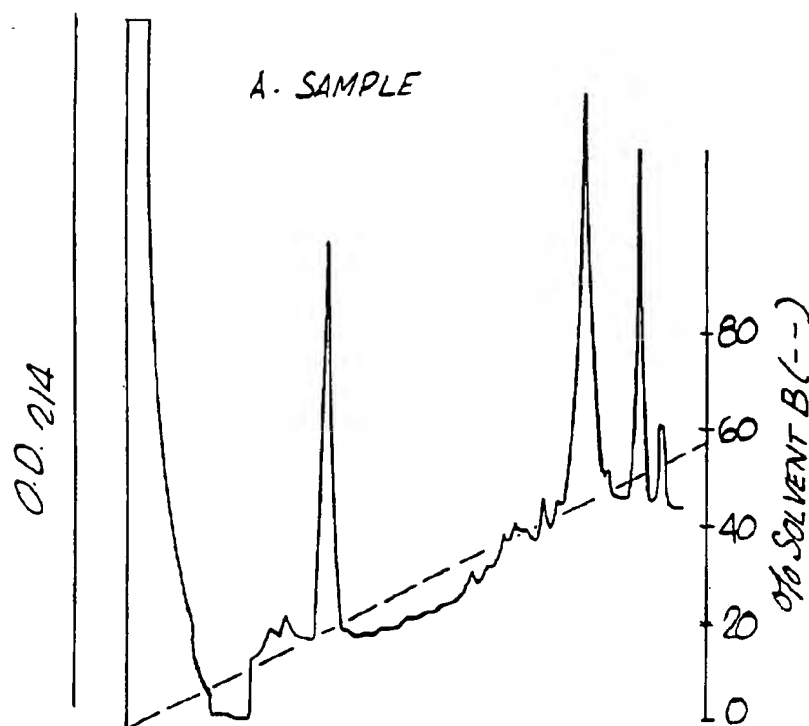


FIG.9  
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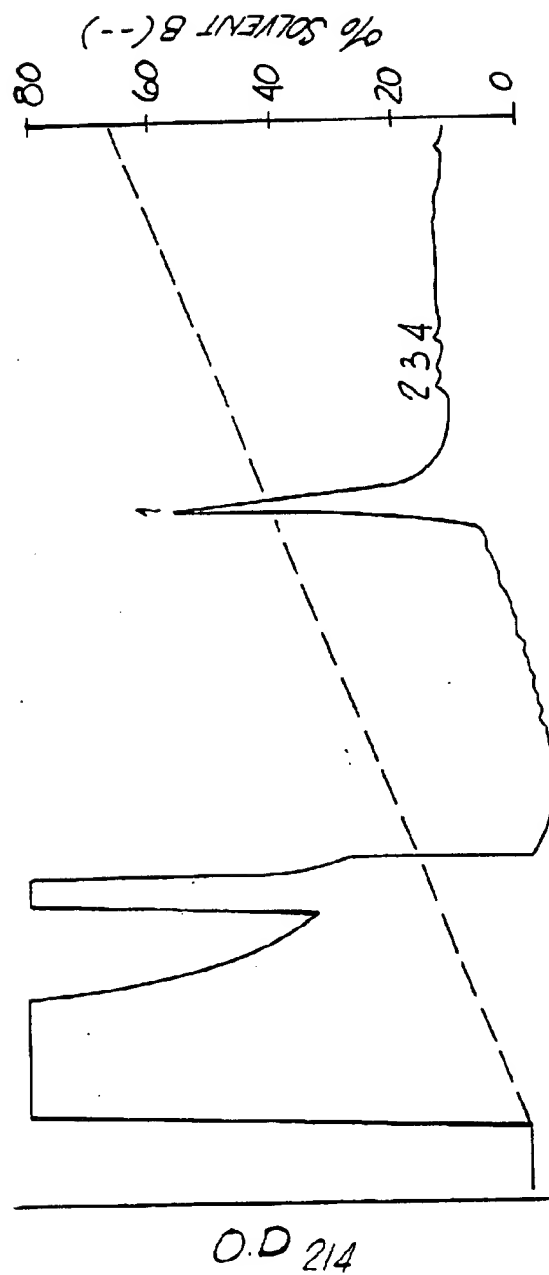


FIG.10

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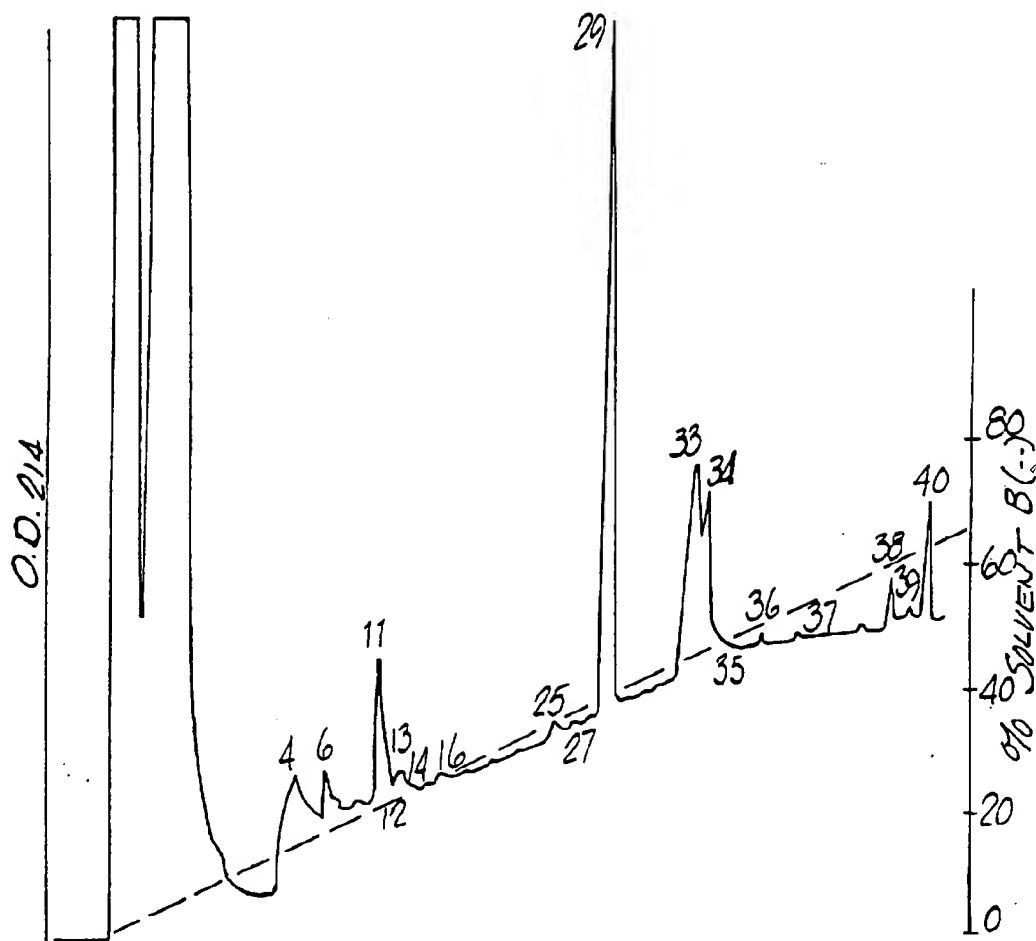


FIG. II

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**FIGURE 12**An internal sequence of the GDNF

Asp-(Lys/Gln)-Ile-Leu-Lys-Asn-Leu-(Gly)\*-(Arg)-(Val)-  
(Arg)-(Arg)-Leu

\*Amino acid residues placed in parentheses are ones identified with less certainty.

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CCCCCGGCT	GCAGGAATTC	GGGG	CTC	TAC	GGA	GAC	CGG	ATC	CGA	GGT	CCC	GCC	GCC	34
			V	Y	G	D	R	I	R	G	A	A	A	
CGA	CGG	GAC	TCT	AAG	ATG	AAG	TTA	TGG	GAT	CTC	GTG	GCT	CTG	88
G	R	D	S	K	M	K	L	W	D	V	V	A	C	
						*								
CTC	CAC	ACC	GCG	TCT	GCC	TTC	CCG	CTG	CCC	GCC	GGT	AAG	AGG	142
L	H	T	A	S	A	F	P	L	P	A	G	K	R	
CCC	GCC	GAA	GAC	CAC	TCC	CTC	GGC	CAC	CGC	CGC	GTG	CCC	TTC	196
P	A	E	D	H	S	L	G	H	R	R	V	P	F	
GAC	TCC	AAT	ATG	CCC	GAA	GAT	TAT	CCT	GAC	CAG	TTC	GAT	GTC	250
D	S	N	M	P	E	D	Y	P	D	Q	F	D	D	
ATT	CMA	GCC	ACC	ATC	AAA	AGA	CTG	AAA	AGG	TCA	CCA	GAT	AAA	304
I	Q	A	T	I	K	R	L	K	R	S	P	D	K	
CTT	CCT	CGA	AGA	GAG	AGG	AAC	CGG	CMA	GCT	GCA	GCT	GCC	AGC	358
L	P	R	R	E	R	N	R	Q	A	A	A	A	S	

FIG.13

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AGA	GGG	AAA	GGT	CGC	AGA	GGC	CAG	AGG	GGC	AAA	AAT	CGG	GGG	TGC	TGC	TTA	ACT	412
R	G	K	G	R	R	G	Q	R	G	K	N	R	G	C	V	L	T	
GCA	ATA	CAC	TTA	AAT	GTC	ACT	GAC	TTG	GGT	TTG	GGC	TAC	GAA	ACC	AAG	GAG	GAA	466
A	I	H	L	N	V	T	D	L	G	L	G	Y	E	T	K	E	E	
CTG	ATC	TTT	CGA	TAT	TGT	AGC	GGT	TCC	TGT	GAA	GGC	GCC	GAG	ACA	ATG	TAC	GAC	520
L	I	F	R	Y	C	S	G	S	C	E	A	A	E	T	M	Y	D	
AAA	ATA	CTA	ANA	AAT	CTG	TCT	CGA	AGT	AGA	AGG	CTA	ACA	AGT	GAC	AAG	GTA	GGC	574
K	I	L	K	N	L	S	R	S	R	R	L	T	S	D	K	V	G	
CAG	GCA	TGT	TGC	AGG	CCG	GTC	GCC	TTC	GAC	GAC	CTG	CTG	TCC	TTT	TTA	GAC	GAC	628
Q	A	C	C	R	P	V	A	F	D	D	D	L	S	F	L	D	D	
AGC	CTG	GTT	TAC	CAT	ATC	CTA	AGA	AAG	CAT	TCC	GCT	AAA	CGG	TGT	GGA	TGT	ATC	682
S	L	V	Y	H	I	L	R	K	H	S	A	K	R	C	G	C	I	
																		745
TGA CCTTGGCTCC AGAGACTGCT GTGTATTGCA TTCTGTCTAC AGTGCGAAGA AAGGGACCAA																		
																		815
GGTTCGCCAGG AATATTTC CCAGAMGGA AGATAAGGAC CAAGAAGGCA GAGGCAGAGG CCGAAGAAGA																		
																		815
AGANGAANAAG AAGGACGAAG GCAGCCNTCT GTGGAGCCT GTAGAAGGAG GCCCAGCTAC AG																		

FIG 13 (CONT)

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FIGURE 14

S	P	D	K	Q	A	A	A	A	S	P	E	N	S
L	P	R	R	E	R	N	R	K	G	C	V	L	T
R	G	X	G	R	R	G	R	L	E	T	K	E	E
A	I	H	L	N	V	T	C	E	A	T	M	Y	D
L	I	F	R	Y	C	S	L	C	A	D	X	V	G
X	I	L	K	N	L	S	P	R	T	F	L	D	D
Q	A	C	C	R	P	V	I	D	L	C	G	C	I
S	L	V	Y	H	I	L	R	S	K	C	G	C	I

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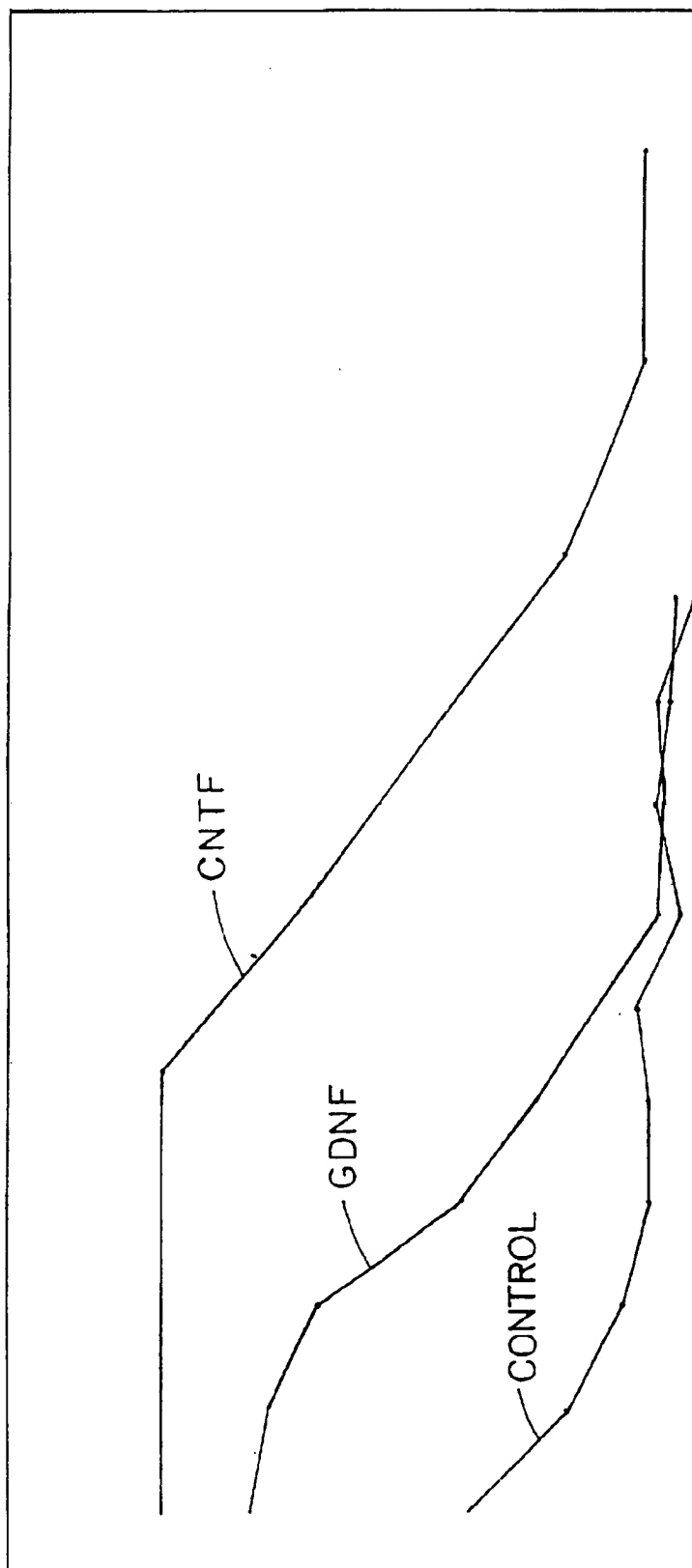


FIG.15

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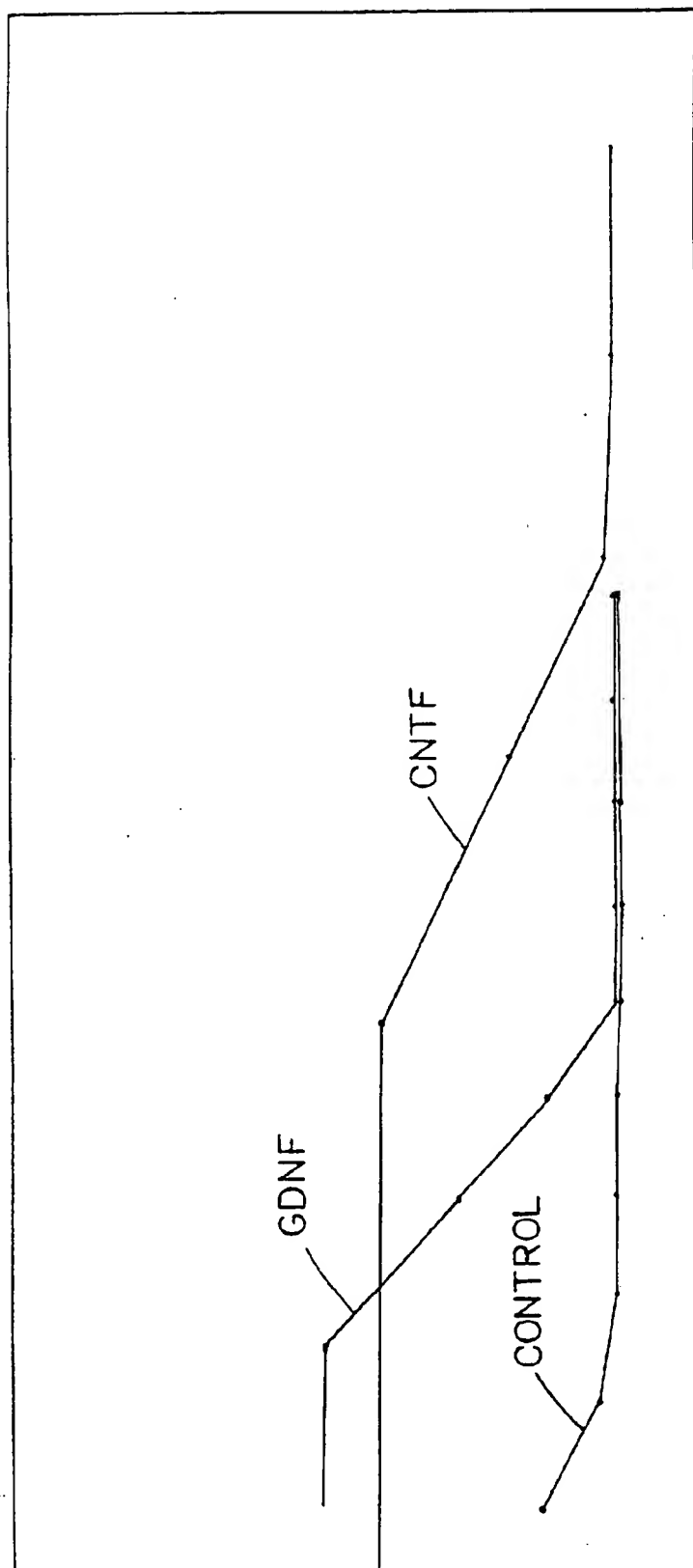


FIG.16

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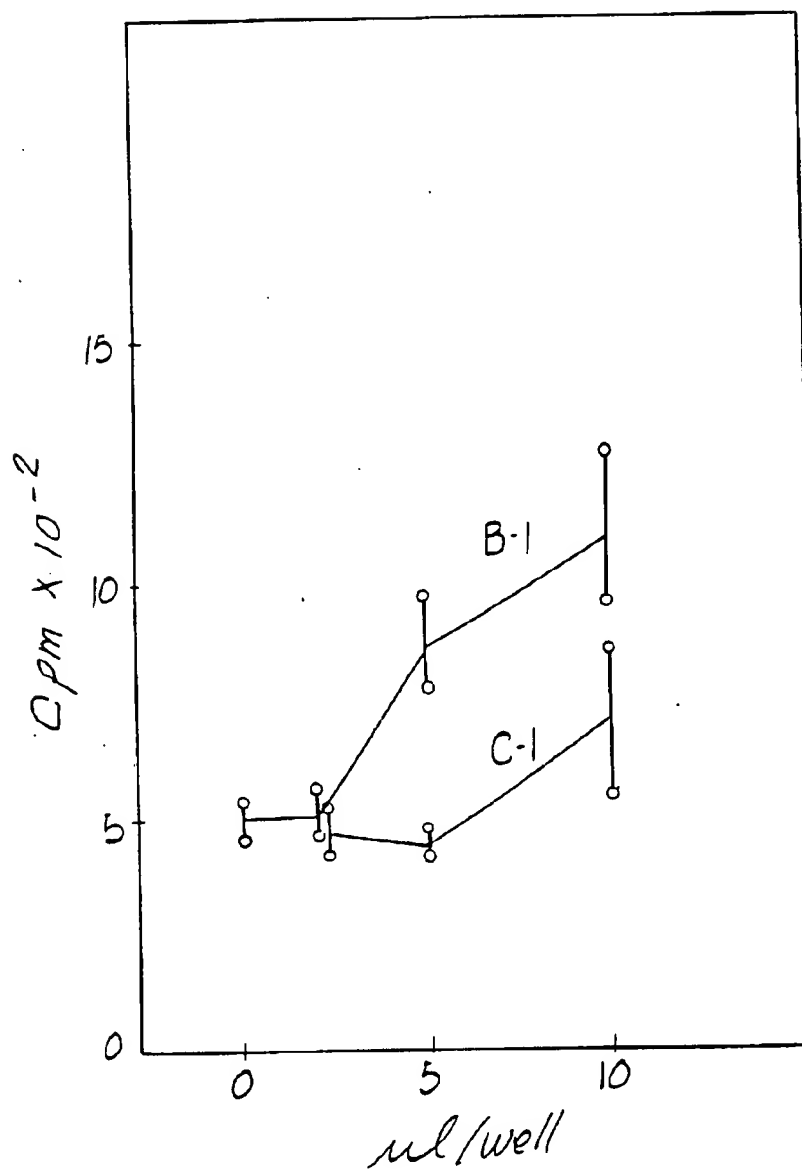


FIG.17

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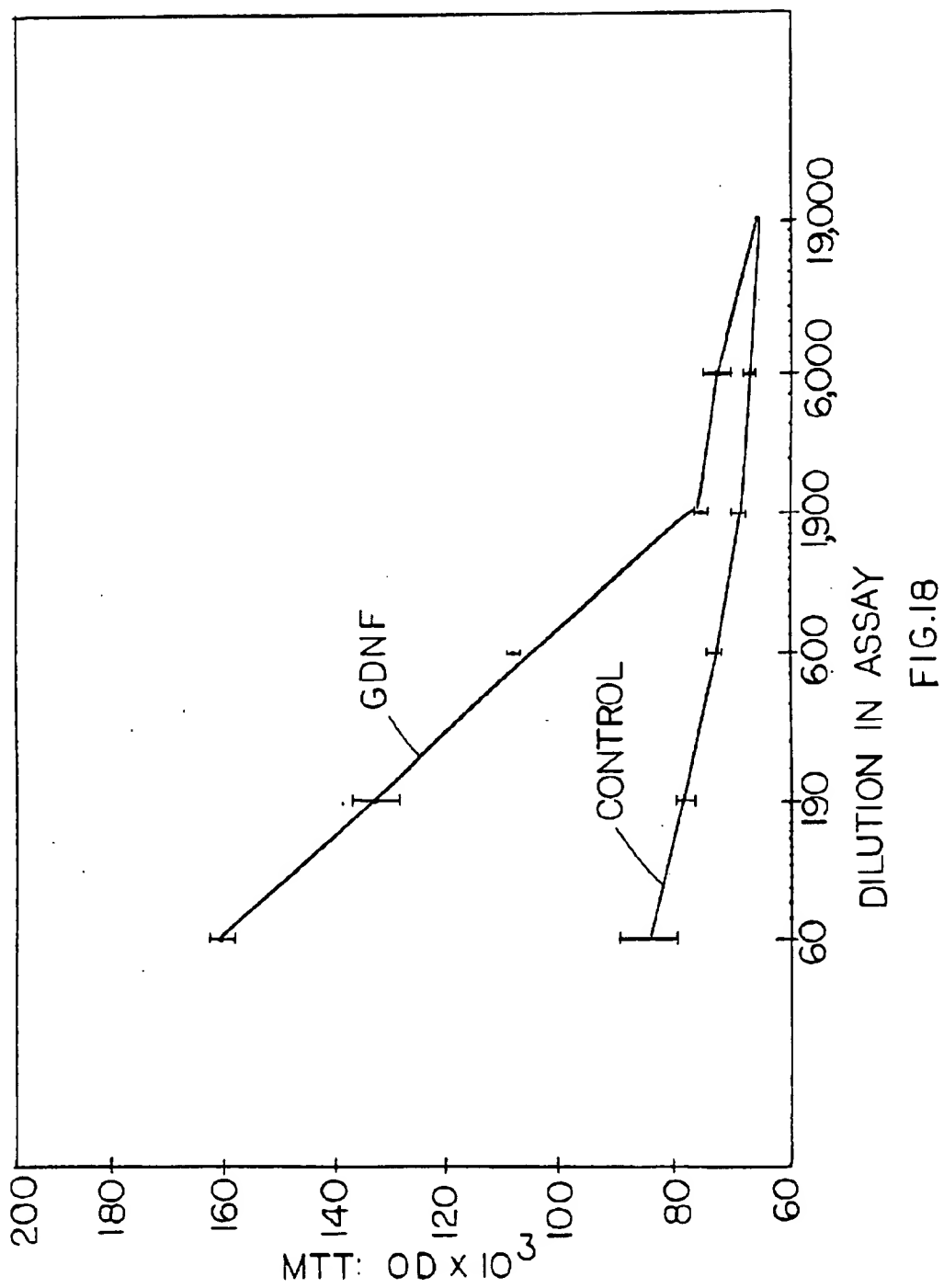


FIG.18

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**SUBSTITUTE SHEET**

[illegible]

<u>GAT</u>	<u>TTT</u>	<u>ATT</u>	<u>CAA</u>	<u>GCC</u>	<u>ACC</u>	<u>ATT</u>	<u>AAA</u>	<u>AGA</u>	<u>CTG</u>	<u>AAA</u>	<u>AGG</u>	<u>TCA</u>	<u>CCA</u>	<u>GAT</u>	<u>AAA</u>	<u>CAA</u>	<u>ATG</u>	<u>GCA</u>	<u>GTG</u>	<u>CTT</u>
D	F	I	Q	A	T	I	K	R	L	K	R	S	P	D	K	Q	M	A	V	L

$\overline{\text{CCT}}$   $\overline{\text{AGA}}$   $\overline{\text{AGA}}$   $\overline{\text{GAG}}$   $\overline{\text{CGG}}$   $\overline{\text{AAT}}$   $\overline{\text{CGG}}$   $\overline{\text{CAG}}$   $\overline{\text{GCT}}$   $\overline{\text{GCA}}$   $\overline{\text{GCT}}$   $\overline{\text{GCC}}$   $\overline{\text{AAC}}$   $\overline{\text{CCA}}$   $\overline{\text{GAG}}$   $\overline{\text{AAT}}$   $\overline{\text{TCC}}$   $\overline{\text{AGA}}$   $\overline{\text{GGA}}$   $\overline{\text{AAA}}$   $\overline{\text{GGT}}$   
 $\text{P}$   $\text{R}$   $\text{R}$   $\text{R}$   $\text{E}$   $\text{R}$   $\text{N}$   $\text{R}$   $\text{Q}$   $\text{A}$   $\text{A}$   $\text{A}$   $\text{N}$   $\text{P}$   $\text{E}$   $\text{N}$   $\text{S}$   $\text{R}$   $\text{G}$   $\text{K}$   $\text{G}$

CGG	AGA	GGC	CAG	AGG	GGC	AAA	AAC	CGG	GGT	TGT	GTC	TTA	ACT	ATA	CAA	TTA	AAT	GTC	ACT
R	R	G	Q	R	G	K	N	R	G	C	V	L	T	A	I	H	L	N	V

GAC	TTG	GST	CTG	GGC	TAT	GAA	ACC	AAG	GAG	GAA	CTG	ATT	TTT	AGG	TAC	TGC	AGC	GGC	TCT	TGC
D	L	G	L	G	Y	E	T	K	E	E	L	I	F	R	Y	C	S	G	S	C

$\overline{\text{GNT}}$   $\overline{\text{GCA}}$   $\overline{\text{GCT}}$   $\overline{\text{GAG}}$   $\overline{\text{ACA}}$   $\overline{\text{ACG}}$   $\overline{\text{TAC}}$   $\overline{\text{GAC}}$   $\overline{\text{AAA}}$   $\overline{\text{ATA}}$   $\overline{\text{TTC}}$   $\overline{\text{AAA}}$   $\overline{\text{AAC}}$   $\overline{\text{TTC}}$   $\overline{\text{TCC}}$   $\overline{\text{AGA}}$   $\overline{\text{AAT}}$   $\overline{\text{AGA}}$   $\overline{\text{AGG}}$   $\overline{\text{CTG}}$   $\overline{\text{CTG}}$   
 $\text{D}$   $\text{A}$   $\text{A}$   $\text{E}$   $\text{T}$   $\text{T}$   $\text{Y}$   $\text{D}$   $\text{K}$   $\text{I}$   $\text{L}$   $\text{K}$   $\text{N}$   $\text{L}$   $\text{S}$   $\text{R}$   $\text{N}$   $\text{R}$   $\text{R}$   $\text{L}$   $\text{V}$

AGT GAC AAA GTA GGG CAG CCA TGT TGC AGA CCC ATC GCC TTT GAT GAT GAC CTG TCG TTT TTA  
S D K V G Q A C C R P I A F D D D L S F L

FIG. 19

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GAT	GAT	AAC	CTG	GTT	TAC	CAT	ATT	CTA	AGA	AAG	CAT	TCC	GCT	AAA	AGG	TGT	GGA	TGT	ATC	TGA
D	D	N	L	V	Y	H	I	L	R	K	H	S	A	K	R	C	G	C	I	.

562

ctcgggtccagagactgctgtgtattgcatttcctgctacagtgcaaaagaag

FIG.19 (CON'T)

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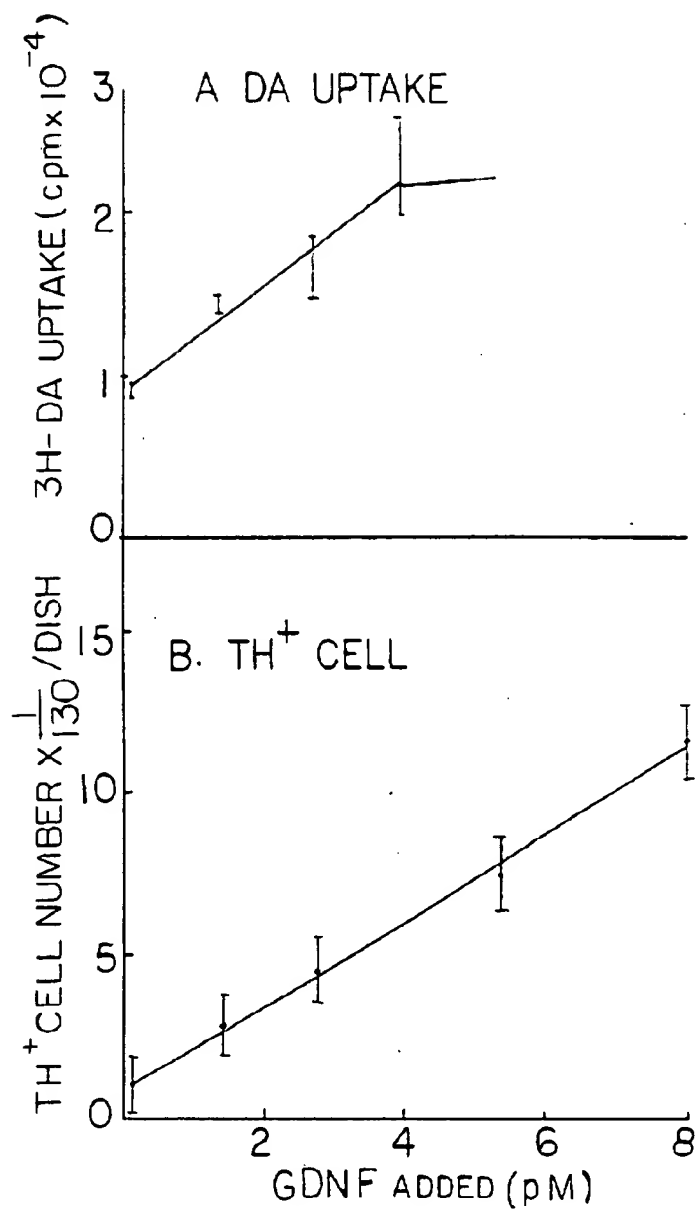


FIG. 20

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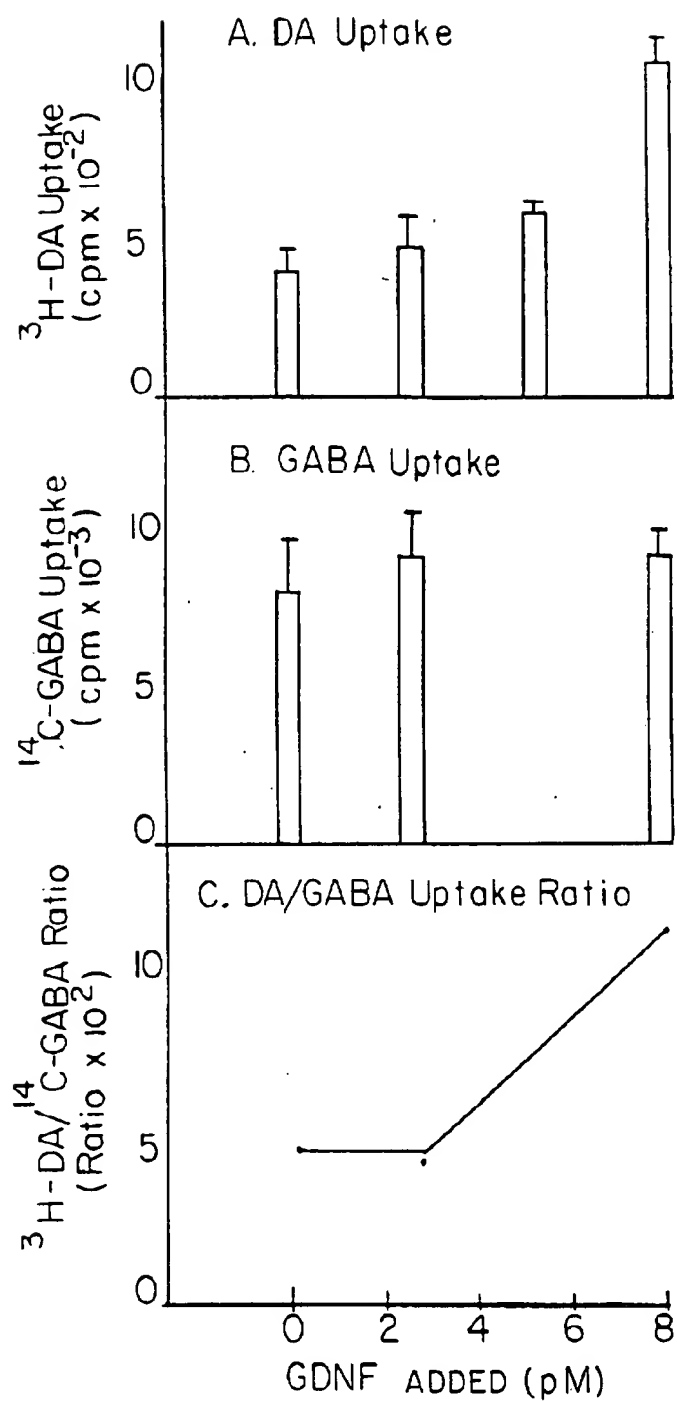


FIG.2I

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4

ttcttccccacctccccgcctgccccgcga  
G A A A A A

97

GGA CGG GAC TTT AAG ATG AAG TTA TGG GAT GTC GTG GCT GTC TGC CTG GTG CTG  
G R D F K M \* L W D V V A V C L V L

151

CTC CAC ACC GCG TCC GCG TTC CCG CTG CCC GGT AAG AGG CCT CCC GAG GCG  
L H T A S A A F P L G A G K R P P E A

205

CCC GCC GAA GAC CGC TCC CTC GGC CGC CGC CGC TTC GCG CTG AGC AGT  
P A E D R S L G' R R R F A L S S

223

GAC Tgtaagaaccggttcc  
D

FIG.22



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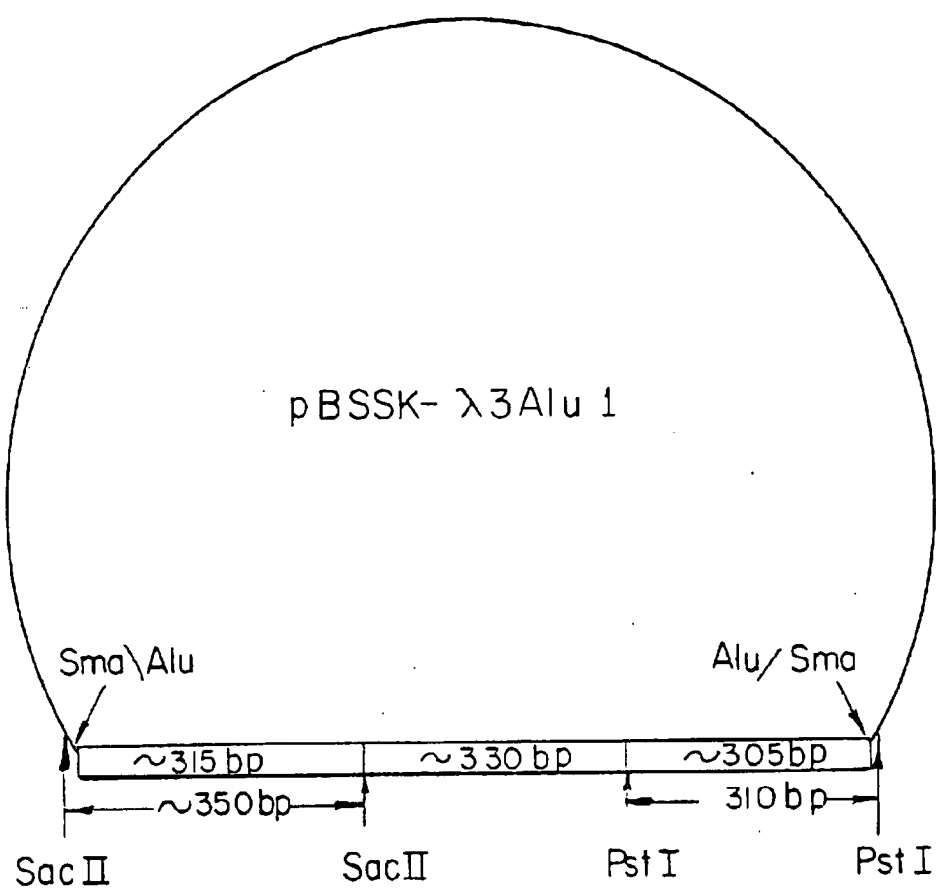


FIG.23

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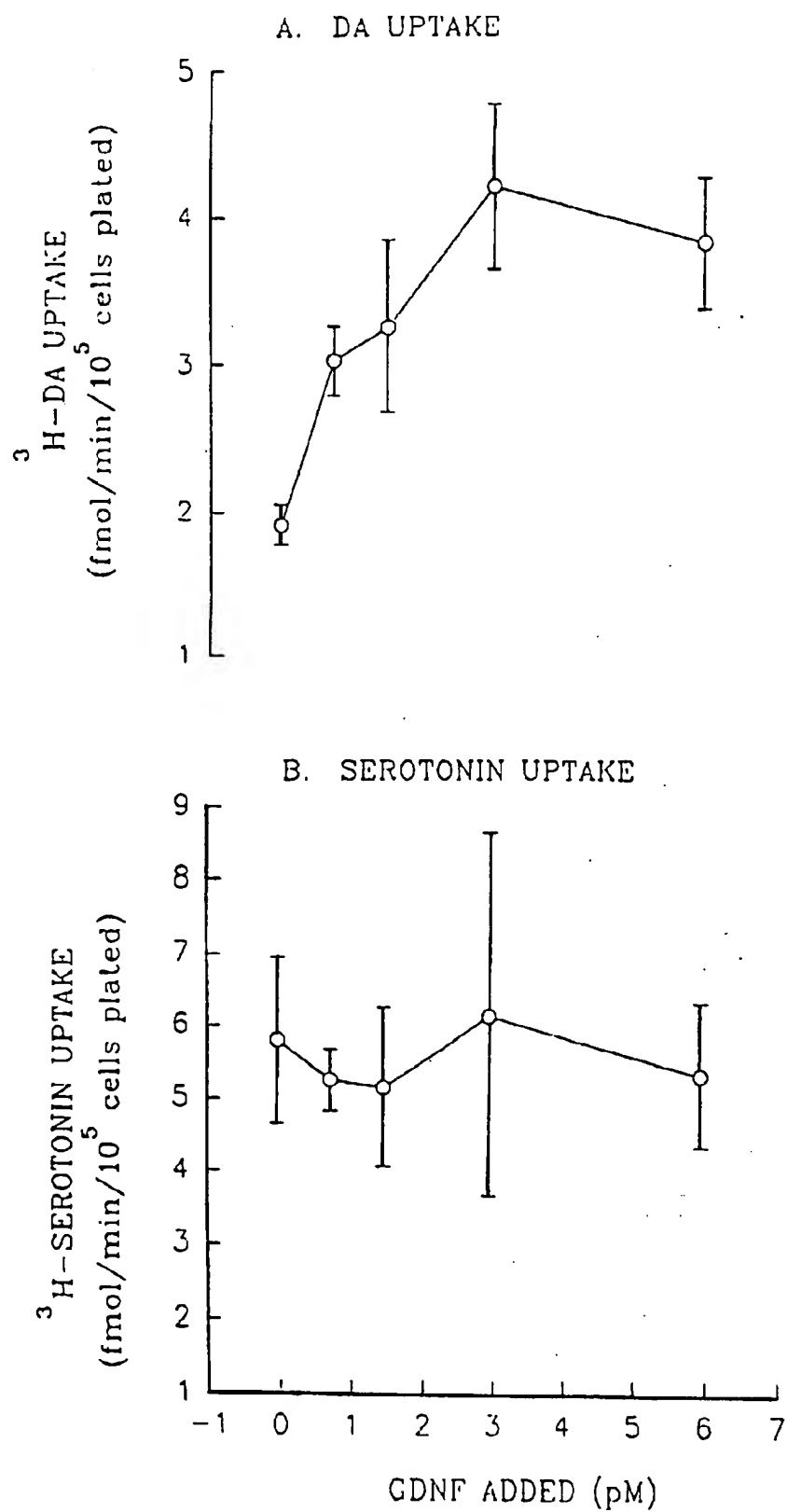


FIG.24

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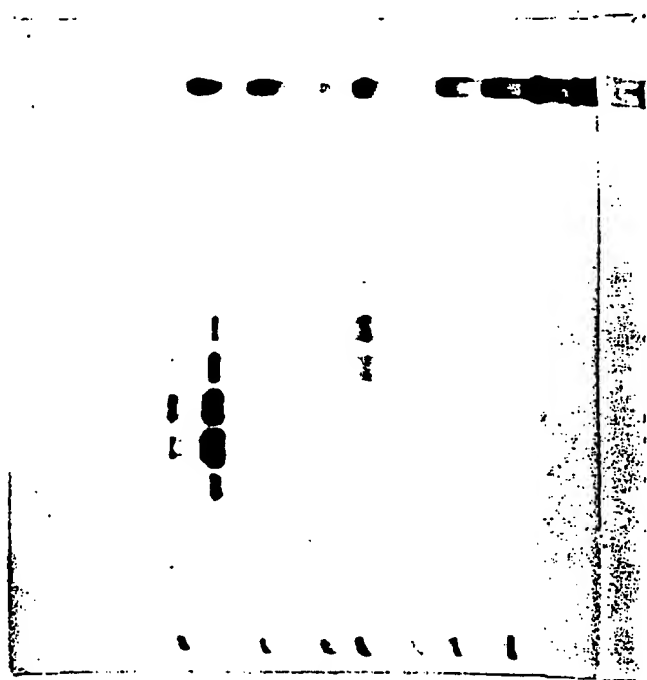


FIG.25

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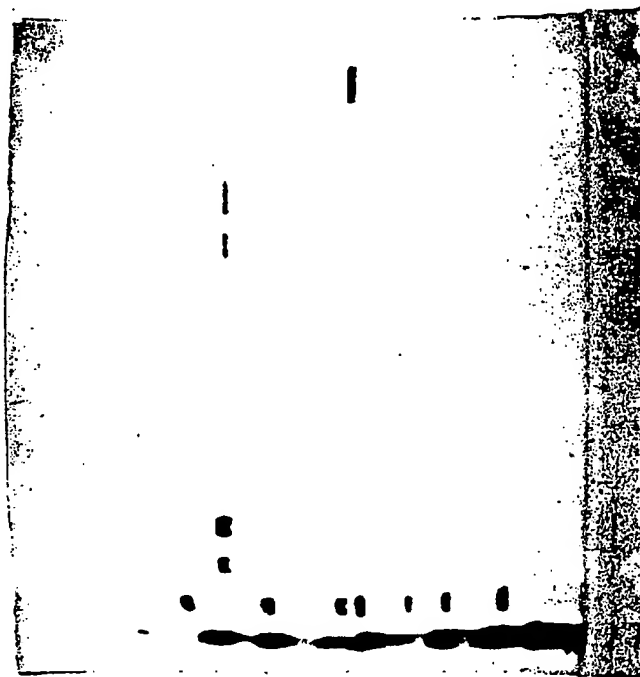


FIG.26

SUBSTITUTE SHEET

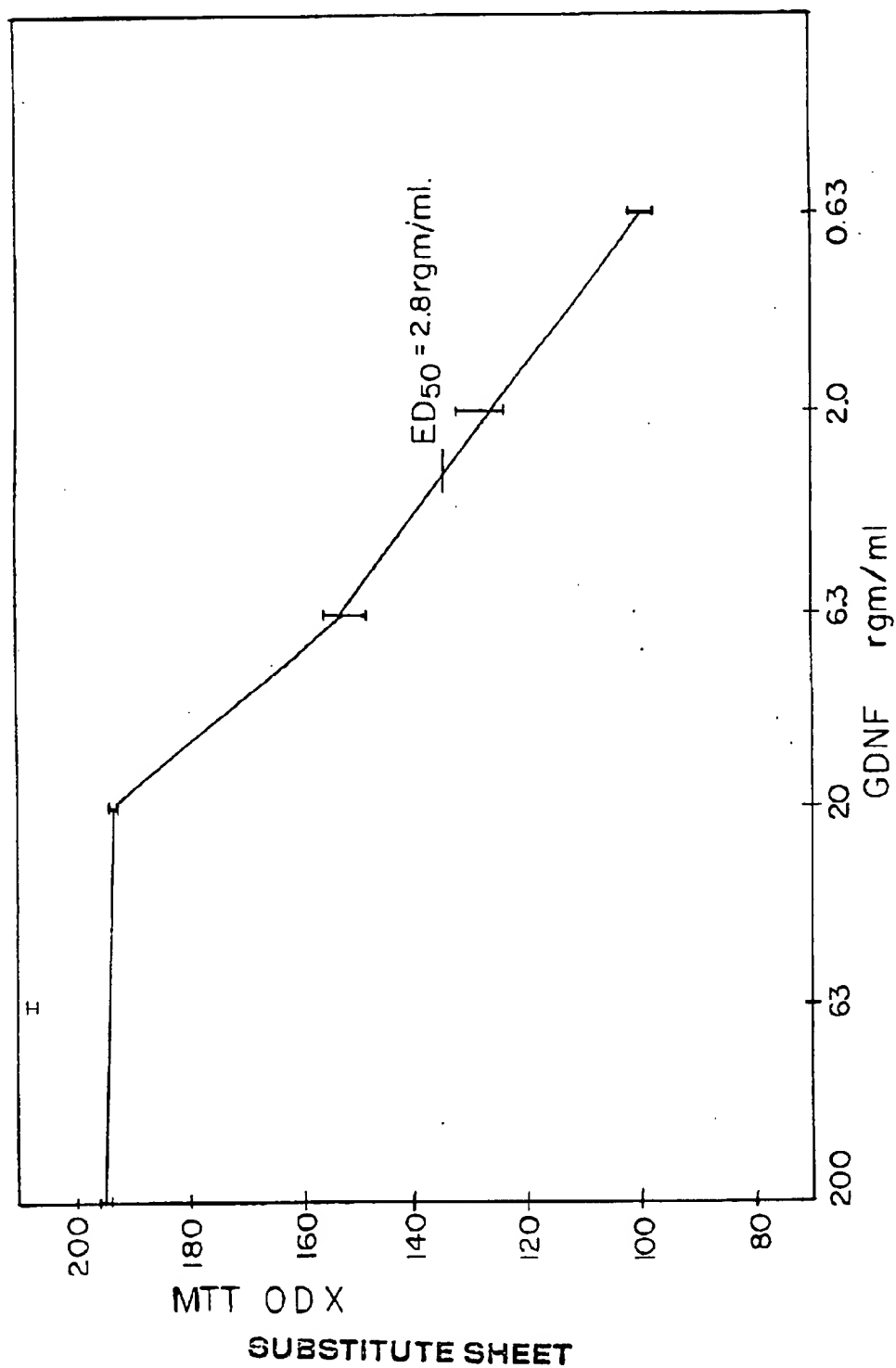


FIG.27

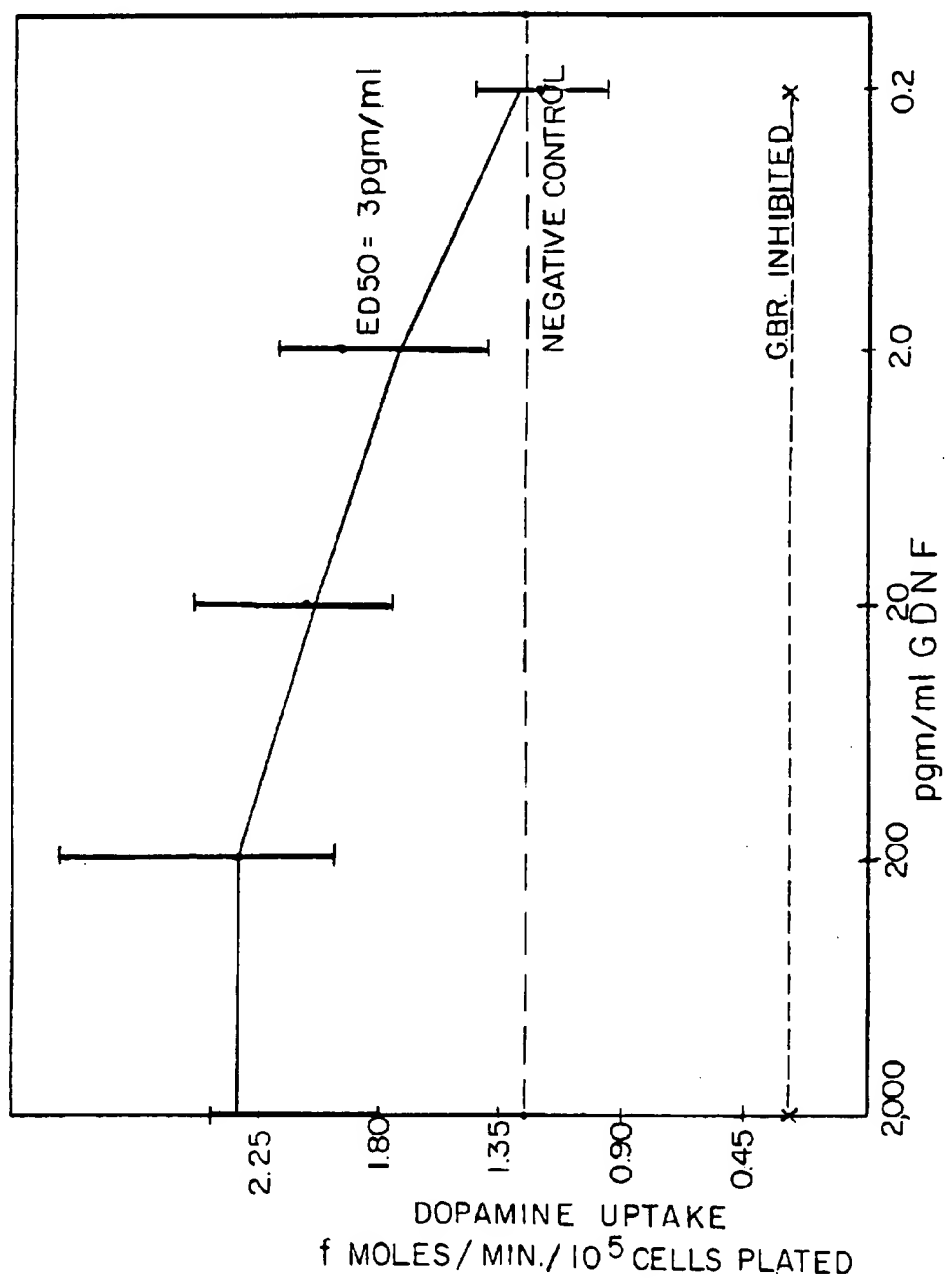


FIG. 28

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## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US92/07888

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : C07H 15/12; C12P 21/06; C12N 1/22; A61K 37/36

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/399, 387, 417; 514/12; 536/27; 435/320.1, 252.3, 69.1; 604/890.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Dialog, search terms: glial cells, glioblastoma, glial-derived, growth factor

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<u>Exp. Cell Res.</u> , Volume 120, No. 1, issued 1980, Norrgren et al., "Release of Nerve Growth Factor By Human Glial Cells in Culture", pages 31-40, see abstract.	1-25, 37-40, 56-74
Y	<u>Bio/Techniques</u> , Volume 1, No. 4, issued 1983, Sofer et al., "Designing an Optimal Chromatographic Purification Scheme for Proteins", pages 198-203, entire document.	1-25
Y	Crichton, "Proteins", published 1984 by Freeman (N.Y.), see pages 39-42	1-25, 37-41, 44-55, 56-59, 60-62
Y	NATURE, Volume 303, issued 30 June 1983, Ullrich et al., "Human beta-Nerve Growth Factor Gene Sequence Highly Homologous to that of Mouse", pages 821-825	26-36, 42-43, 63-69, 44-55, 70-74
Y	BRAIN RESEARCH, Volume 560, issued 1991, Bakhit et al., "Increase in Glia-Derived Nerve Growth Factor Following Destruction of Hippocampal Neurons", page 76-83.	1-25, 37-41, 56-59, 60-62, 70-74, 63-69

☐ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

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*A* document defining the general state of the art which is not considered to be part of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

09 December 1992

Date of mailing of the international search report

13 JAN 1993

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